In the Specification

Kindly amend the last paragraph on page 12 as follows:

~~Fig. 18 shows~~ Figs. 17E, 17F, 17G and 17H show ribosomal fractionation of *E. coli* JM83 cells transformed with pINZ or pINZDB1. Ribosomal particles were isolated as described by Dammel and Noller, 1995. Cultures of *E. coli* JM83 cells carrying pINZ or pINZDBl were grown at 37°C in LB medium containing 50 ~~mg~~ µg/ml of ampicillin. At mid-log phase (OD_{600}=0.4) 1 mM of IPTG was added to each culture. Chloramphenicol (0.1 mg/ml) was added at 15, 30 and 60 min after IPTG addition. The cell extracts were then layered on top of a 5-40% (w/w) sucrose gradient. The polysomes and ribosomal subunits were separated by centrifugation at 151,000 x g for 2.5 hr at 4°C. The polysome profiles were then detected by using a FPLC system. 0.2 ml from each fraction (0.5 ml) were spotted on a Nitrocellulose membrane using the Minifold II Slot-Blot System (Schleicher and Schuell). The *lac*Z mRNA was detected by hybridization using the [^{32}P]-labeled M13-47. (See Fig. 17E) Phosphorimager values from the hybridization are plotted ~~at the right side~~ in Figs. 17F, 17G and 17H. The pINZ and pINZDBl mRNAs are shown in closed and open squares, respectively.

Kindly amend the three consecutive paragraphs spanning pages 13 and 14 as follows:

Fig. ~~19.~~ 18 shows translational enhancement by a perfectly matching DB at 37°C. (A) Estimation of pINZ and pINZDB1 mRNAs. Cultures of *E. coli* JM83 carrying pINZ or pINZDBl were grown at 37°C under the same conditions described in Figure 4. Total RNA extracted at 15, 30 and 60 min after IPTG (1 mM) addition was used as a template for primer extensions according to the procedure described previously. (B) β-Galactosidase activity of pINZ and pINZDBl in multi-copy expression system. *E. coli* JM83 cells transformed with pINZ or pINZDB1 were grown at 37°C under the same condition described in Figure 20A. β-Galactosidase activity was measured before (time 0) and 0.5, 1, 1.5, 2 and 2.5 hr after IPTG (1 mM) addition (open circles and squares). Closed circles and squares represent the activities in the absence of IPTG.

Fig. ~~20~~ 19 shows cell-free synthesis of β-galactosidase from pINZ and pINZDB1. (A) pINZ or pINZDBl DNA (160 ng; 1 µl) was added to the *E. coli* 30S extract (20µl) (Promega) and the transcription-translation coupled reaction was carried out. Lane 1, pINZ DNA; lane 2, pINZDB1 DNA; and lane 3, a control reaction without added DNA. Samples were precipitated with

acetone and analyzed by 15% SDS-PAGE to detect the production of β-galactosidase. (B) Time course *in vitro* synthesis of β-galactosidase from pINZ and pINZDB1 was carried out as described above. Samples were taken after 15, 30, 60 and 120 min incubation at 37°C. (C) Each reaction from the time course experiment described above was done in duplicate with non radioactive methionine, spotted on nitrocellulose membrane and hybridized with [^{32}P] labeled M13-47 oligonucleotide.

Fig. ~~22~~ 20 shows translational enhancement of pINZDB1 in cells with S2-depleted ribosomes (A) β-galactosidase activity from pINZ and pINZDB1. *E. coli* CS240 and CS239 (Shean and Gottesman, 1992) were transformed with pINZ or pINZDB1 and cultures were grown at 30°C in LB medium. At mid-log phase the cells were shifted to 42°C in the presence of 1 mM of IPTG. β-Galactosidase activity was measured as Miller units before (time 0) and at 0.5, 1, 1.5, 2.5 and 3.5 hr after shift to 42°C. (B) Relative induction of the *lacZ* expression between pINZDBl and pINZ in cells with S2-depleted ribosomes. Before the shift to 42°C (time 0) the ratio of the (β-galactosidase expression from pINZ and pINZDBl in CS239 to that of CS240 was estimated as 1, and the ratios after the shift to 42°C were calculated accordingly.

Kindly amend the paragraph spanning pages 62 and 63 as follows:

In order to examine whether DB enhances translation initiation we next analyze the ability of *lacZ* mRNA from pINZ and pINZDB1 to form polysomes. For this experiment, *pcnB*$^{+}$ cells were used to amplify the effect DB. Interestingly, cells with pINZDB1 could not form colonies on LB plates in the presence of 1 mM IPTG, while cells with pINZ formed colonies. The lethal effect of IPTG on the cells with pINZDB1 is considered to be due to overexpression of β-galactosidase. After the addition of IPTG, cell growth was stopped by the addition of chloramphenicol (0.1 mg/ml) at 15, 30 and 60 min and then polysome profiles were examined as shown in Fig. ~~17D~~ 17E. From each gradient fraction (500 ml), 200 ml were spotted on a nitrocellulose membrane and the amount of the *lacZ* mRNA analyzed using a 24-base antisense oligonucleotide (M13-47 oligonucleotide). The amounts of the *lacZ* mRNA were quantified by a phosphorimager and are displayed in ~~Fig. 17D~~ Figs. 17F, 17G and 17H. While the polysome profiles are similar, there are significant differences in the distribution of the *lacZ* mRNA; at 15 min the *lacZ* mRNA mainly exists in the upper half of the gradient (fraction 8 to 14, corresponding to 70S to 30S ribosomes) with pINZ, while with pINZDB1 a major peak (fraction

3 to 8) is formed in the lower half of the gradient. At 30 min, the *lacZ* mRNA from pINZ moved to the position of 70S ribosome, while the *lacZ* mRNA from pINZDBI maintained a similar pattern as that at 15 min. At 60 min a major fraction of the *lacZ* mRNA from pINZ remained in the upper half of the gradient, while the *lacZ* mRNA from pINZDB1 was broadly distributed from higher order polysomes to 70S ribosome fraction. Therefore, the reason why cells harboring pINZDB1 could not form colonies on LB plates containing 1 mM IPTG may be due to a decrease in the concentration of free ribosomes as a result of the massive expression of a highly translatable DB-containing mRNA (Vind *et al.*, 1993). These results indicate that DB enhances the efficiency of polysome formation probably due to a translation initiation enhancement.